REMARKS

Claims 1-41 have been canceled without prejudice. Claims 42, 48, 50 and 53 have been amended. Support for the amended claims can be found throughout the specification, claims and figures as originally filed. No new matter has been added by amendments to the claims.

Applicant now turns to comments made by the Examiner in the Office Action as follows.

1. Claims 42-67 are newly rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

> The Examiner states, "Claims 42-58 are drawn to cells. This includes naturally occurring cells that have not seen the "hand of man." Using the words, "recombinant," "in vitro," or "isolated" to describe the cells would overcome the rejection. Claims 59-67 depend on claims 42-58 and are thus included in the rejection.".

Claims 42, 48, 50 and 53 have been amended to include a limitation directed to "*in vitro*" systems, thereby obviating the bases for rejection.

2. Claims 42-67 remain rejected in modified form under 35 U.S.C. 112, first paragraph.

> The Examiner states, ..." while being enabling for
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> 1) an isolated mouse or human muscle cell or a cell line comprising a disruption in its endogenous CIC-1, and a cell membrane preparation or cell vesicle obtained from said cell or cell line,
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> an isolated mouse or human cell or a cell line obtained from the thick ascending limb of Henle loop, the distal convoluted tubule, the acid-transporting intercalated cells of the collecting duct, and epithelial cells of the inner ear, wherein said cell or cell line comprises a disruption in its endogenous CIC-Kb, and a cell membrane preparation or cell vesicle obtained from said cell or cell line,

an isolated mouse or human cell or cell line obtained from the proximal tubule of the kidney comprising a disruption in its endogenous CIC-5, and a cell membrane preparation or cell vesicle obtained from said cell or cell line, and

an isolated mouse or human osteoclast or osteoclast cell line comprising a disruption in its endogenous CIC-7, and a cell membrane preparation or cell vesicle obtained from said osteoclast or osteoclast line

does not reasonably provide enablement for

1) any cell, cell line, or cell membrane preparation, or cell vesicle comprising:

a) any disruption in CIC-2,

b) any disruption in CIC-3,

c) any disruption in CIC-Ka,

d) any disruption in CIC-4,

e) any disruption in CIC-6,

f) any multiple disruptions in CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-4, CIC-5, CIC-6, or CIC-7,

g) any overexpression of CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-4, CIC-5, CIC-6, or CIC-7,

2) any method of using any cell, cell line, or cell membrane preparation, or cell vesicle comprising:

a) any disruption in CIC-2,

b) any disruption in CIC-3,

c) any disruption in CIC-Ka,

d) any disruption in CIC-4,

ed) any disruption in CIC-6,

f) any multiple disruptions in CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-4, CIC-5, CIC-6, or CIC-7

g) any overexpression of CIC-1, CIC-2, CIC-Ka, CIC-Kb, CIC-3, CIC-4, CIC-5, CIC-6, or CIC-7.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

At the time of filing, the art teaches that a mutation in the CIC-1 gene results in myotonia in humans (Groneimeier et al., 1994, JBC, 269: 5963-5967, also Nilius and Droogmmans, 2003, Acta Physiol. Scand., 177: 119-147, Table 2), the disruption of CIC-Kb results in hypokalaemia, elevated serum bicarbonate levels, salt-wasting, and dehydration resulting from a failure of normal transit of chloride across the basolateral membrane into the bloodstream and deafness (Simon et al, 1997, Nature Genetics, 17: 171-178, previously cited), the disruption of CIC-5 results in Dent's disease characterized by kidney stones, nephrocalcinosis, rickets, and renal failure (Gunter et al., 1998, PNAS, USA, 95: 8075-8080, previously cited), and disruption of CIC-7 in mouse results in osteopetrosis (Kornak et al., 2001, Cell, 104: 205-215). In the case of CIC-Ka, no known human mutations have been reported (Jentsch et al., 2005, 115: 2039-2046, page 2042, 1st col., 3rd parag.); in the case of CIC-2, CIC-3, CIC-4, CIC-6, no known human disease or disorder has been associated with these channels (Nilius and Droogmans, Table 2). While the art teaches that homologous recombination, as a method of generating targeted disruptions in a gene of interest in a genome, is well known, the art teaches that phenotypes exhibited by the mice associated with the gene disruption are not predictable. At the time of filing, the art did not consider the phenotype of a knock-out or transgenic mouse to be predictable. In addition, the art did not consider the correlation between any observed mouse phenotypes and human disease phenotypes as predictable. Doetschmann et al. teaches that "[o]ne often hears the comment that genetically engineered mice, especially knockout mice, are not useful because they frequently do not yield the expected phenotype, or they don't seem to have any phenotype" (Doetschmann, 1999, Lab. Animal Sci., 49: 137-143, see page 137, 1st col., 1st parag.). Doetschmann provides numerous examples of instances in which genes considered well-characterized *in vitro* have produced unexpected phenotypes or indiscernible or no phenotypes in transgenic or knockout mice. Moens et al. further teaches that different mutations in the same gene can lead to unexpected differences in the phenotype observed. Moens et al. shows that two mutations produced by homologous recombination in two different locations of the **N**-myc gene produce two different phenotypes in mouse embryonic stem cells, one leaky and one null (Moens et al., 1993, Development, 119: 485-499). Further, the art demonstrates the unpredictability of making a mouse model for human

disease by disrupting the murine gene. Jacks et al. teaches that although retinoblastoma (Rb) gene mutations in humans are associated with retinal tumors, Rb gene knockout mice had tumors in the pituitary gland rather than the retinas (Jacks et al., 1992, Nature, 359: 295-300). Likewise, whereas HPRT deficiency in humans is associated with Lesch-Nyhan syndrome, a severe neurological disorder, HPRT-deficient mice are phenotypically normal (Kuehn et al., 1987, Nature, 326: 295-298 and Jaenisch, 1988, Science, 240: 1468-1474). Thus, the art at the time of filing clearly establishes the unpredictability of determining the phenotype of transgenic or knockout mouse even when the activity of the gene has been extensively studied *in vitro,* and further establishes the unpredictability of generating a mouse model for human disease based on the activity of the gene in humans.

In addition to the phenotypes of knockout mice being unpredictable, the art teaches that while the promise of gene targeting had been to reveal the *in vivo* function of a gene of interest, the functional relevance of gene targeting has been questioned because the mutation might lead to an avalanche of compensatory processes (up- or downregulation of gene products) and resulting secondary phenotypical changes. Thus, a null mutant organism might not only lack the produce of a single gene, but might also possess a number of developmental, physiological, or even behavioral process that have been altered to compensate for the effect of the null mutation (Gerlai, 1996, Trends Neurosci, 19: 177-181, page 177, 1st col., 1st parag.). Gerlai teaches an example wherein background genotype can confound the exhibited phenotypes. Targeted disruption of a gene of interest, α, might lead to changes in expression of alleles b and B for gene β. A regulatory change in gene β might lead to different phenotypic changes, depending on which allele (b or B) is present in the organism with the null mutation in gene *a.* The upshot of this problem is that due to this polymorphism in the genetic background, one cannot conclude for certain that a phenotypic change exhibited in a null-mutant mouse resulted from the null mutation or to the genetic background (Gerlai, page 177, 1st col., under "Polymorphism in the genetic background might make the results of gene-targeting studies difficult to interpret").

In addition to these issues, Racay, 2002, Bratisl Lek Listy, 103: 121-126, teaches that:

"mutations of some genes led to phenotype showing severe defects,-which did not correspond to any clinically important disorder, indicating either high *in vivo* stability of the gene or the interspecies differences. From the view of human medicine, the differences among the species (it means the differences in genetic background, gene expression, metabolism, and

signal transduction) represent the main limitation of the use of genetically modified animals as models of human diseases. Therefore some results acquired by this approach can not be applied in human medicine because of the differences between rodents and human beings (Racay, page 124, under point 5)."

As such, while the knockout mouse may exhibit a phenotype, the phenotypes of the knockout mouse are not necessarily readily applicable to the human condition. Subsequently, the mouse has no apparent use or application. This issue is seen in the chloride channel field. Mice comprising a disruption in CIC-2 exhibit degeneration in the testis and retina, no known human disease directly connected with CIC-2 have been reported (Nilius and Droogmans, page 123, 2nd col., 1st parag. and Table 2). A disruption in CIC-K1 in mice results in nephrogenic diabetes insipidus, while a disease in humans for the homologous gene, CIC-Ka, is not known (Jentsch et al., page 2042, 1st col., 3rd parag. and Nilus and Droogmans, Table 2). Similarly, while the mouse comprising a disruption in CIC-3 exhibits degeneration of the hippocampus and retina, no known human condition has been associated with CIC-3 (Nilus and Droogmans, Table 2). Because there is no known human condition similar to what is seen in the knockout mouse models, an artisan does not know how to use any of the cells obtained from the transgenic mouse comprising a disruption in CIC-2, CIC-K1, or CIC-3.

It is understood that the claims are drawn to cells and not to knockout animals. However, the ability to use the claimed cells stems from whether an artisan could use the cells in the context that a relationship is known between the gene of interest and the human disease or condition. That is, it is not enough to say that minimally, the claimed cells could be used in general studies to determine the pH or electrophysiological status of a cell. Further, the general function of a chloride channel, which allows chloride ions to pass through a cellular membrane, does not provide guidance for an artisan to use specific channels (e.g. CIC-1 versus CIC-5) in the context for which there is a relationship with a human disease. The enabled use of the claimed cell hinges upon the fact that the cell in an *in vitro* assay stems from the understanding what biological characteristics the specific chloride channel has with pathology of a human disease. Subsequently, because CICI-4 and CIC-6 have no known pathology associated with them (e.g. see Nilus and Droogmans, Table 2), the use of cells comprising disruptions in these channels is not enabled.

That said, because the use of the cells depends on the disease, an artisan would know how to use cells obtained from tissues or organs associated with the human disease. In the case of CIC-1, skeletal muscle

is affected (Nilius and Droogmans, page 140, 1st col., 2nd parag.). In the case of CIC-Kb, the thick ascending limb of Henle loop, the distal convoluted tubule, the acid-transporting intercalated cells of the collecting duct in the kidney, and epithelial cells of the inner ear are affected (Jentsch, page 2041, 1st col., 2nd parag. under "CIC-K/barttin: basolateral chloride channels in kidney and inner ear epithelia"). In the case of CIC-5, CIC-5 and cells in the proximal tubule of the kidney have been demonstrated to have a relationship during the disease (Jentsch, page 2042, 2nd col., 2nd parag. under "CIC-5, endocytosis, and Dent disease"). In the case of CIC-7, there is a relationship between CIC-7 and osteoclasts (Jentsch, page 2044, 2nd col., parag. under "CIC-7: role in osteopetrosis"). As this applies to the instant invention, the use of the cells comprising a specific disruption in a chloride channel depends upon which cells are implicated in the human disease or disorder. It is noted that in light of the teachings of Racay, above, the scope of the cells is limited to human and mouse cells, as an artisan cannot predictably arrive at cells obtained from other animals.

With regard to the issue that the claimed invention is not enabled for cells comprising transgene constructs overexpressing any chloride channel(s) of interest, as neither the art or specification provide any guidance for an artisan to arrive at the claimed invention. While it is understood that the methods of transgenesis and transfection are well known in the art, the issue at hand is whether or not an artisan is enabled to use the claimed invention. At the time of filing nothing in the art or the specification teaches any human condition associated with the overexpression of any chloride channel. Further, at the time of filing, the art teaches that overexpression of a gene of interest does not necessarily result in an animal with a predictable phenotype. For example, Duff et al., 1996, Nature, 383: 710-713 teach that while transgenic mice expressing mutant presenilin1 (M146V) exhibit an increase in brain Aβ42 plaque formation, transgenic mice that express wild type presenilin 1 (PSI) do not exhibit any increase in brain Aβ42 plaque formation (Duff et al. page 711, 1st col., 3rd parag., lines 3-6). Duff et al.'s results teach that an artisan cannot predict that overexpression of any transgene would necessarily result in a phenotype. Alternatively, the art teaches that transgenic mice expressing full length human apo B100 mRNA does not significantly edit human apo B mRNA despite normal editing of endogenous mouse apo B mRNA. Davidson concludes that there may be some species specificity to apo B RNA editing (Davidson, U.S. Patent, 5,434,058, patented July 18, 1995, col. 17, 5th parag.). Thus, the art teaches that one cannot predict that a heterologous mRNA can be processed in a transgenic animal. As these issues apply to the instant invention, an artisan cannot predict that overexpression of a gene of interest results in a transgenic animal exhibiting any phenotype and whether that transgenic animal is a model

for a human disease or condition. As such, an artisan cannot obtain a cell from any transgenic animal overexpressing a chloride channel of interest. Further, an artisan is not enabled for the use of any chloride channel in any cell. Similar to the issues concerning cells comprising a disruption in a chloride channel of interest, it is not enough to say that minimally, the claimed cells could be used in general studies to determine the pH or electrophysiological status of a cell. Again, a general function of a chloride channel does not necessarily enable an artisan to practice the claimed invention as it pertains to specific chloride channels. As such, an artisan is not enabled to use any cell overexpressing any chloride channel.

Regarding the issue that the claimed cells comprise multiple overexpression constructs and/or disruptions of chloride channels of interest, the art teaches unpredictability in arriving at the claimed invention. First, regarding the issue of multiple overexpression constructs in cells, the art teaches that an artisan cannot predict what overall phenotype occurs following the expression of multiple transgenes. Siegel et al., 2000, Bioessays, 22: 554-563 teach that mice comprising two transgenes do not necessarily exhibit phenotypes that are the simple combination or an additive result of the two transgenes. Siegel et al. teach that in some cases, mice comprising two transgenes exhibit a phenotype resulting from a synergistic interaction between the two transgenes (Siegel et al., page 559, 2nd col., 1st parag.). Alternatively, Siegel et al. teach that some transgene combinations result in mice that fail to develop mammary tumors (Siegel et al., page 559, 2nd col., 3rd parag.), while single transgenic mice did. As this relates to the instant invention, an artisan cannot predict what phenotype of a disease would result in cells expressing multiple transgenes and thus, an artisan is not enabled to use the claimed cells in any assay. Second, regarding the issue of cells comprising multiple gene disruptions, the art teaches that an artisan cannot predict what phenotypes would result from mice comprising two or more targeted disrupted genes of interest. The art teaches that the effects of gene disruption are not additive. For example, a disruption of biglycan and fibromodulin resulted in mice that exhibited a bone phenotype that was more severe that the phenotype exhibited by mice comprising a single disruption of the gene. These results indicated that the phenotype resulted from a synergistic effect of the two gene disruptions (Young et al., 2003, Glycoconjugate Journal, 19: 257-262, page 260, 2nd col., 3rd parag.). As this applies to the instant invention, an artisan cannot predict that cells comprising multiple targeted gene disruptions would necessarily result in a cell with a predictable phenotype, wherein the cell has in vitro applications as a model for a disease. Third, in light of these issues of unpredictability of phenotype in making cells expressing a transgene or making cells comprising a disruption of a gene of interest, an artisan cannot reasonably predict how to make cells comprising a combination of disruptions and

overexpression constructs, wherein the resulting cell is an in vitro model of disease. It should be pointed out that with regard to these cells comprising multiple chloride channel disruptions and/or multiple transgene constructs that express chloride channels, nothing in the specification or the art provide any guidance that certain combinations of gene disruptions and/or overexpression of chloride channels result in any human disease or disorder. It would be undue experimentation to make any kind of cell comprising gene disruptions and/or transgene overexpression constructs and correlate them with a specific human disease.

Because the claimed cells are not enabled, the method of using them to screen for substances are also not enabled.

The claimed invention encompasses cells that "do not express" or express only to a "reduced functional extent" (e.g. see claim 44) specific chloride channels. The Examiner had previously indicated that these terms encompass a wide breadth of mechanisms that could be used to achieve this state in the claimed cells and has also indicated that the art teaches that aspects of these mechanisms encompassed by the claims are unpredictable and not routine in the art (Office Action, May 19, 2005, pages 13-16). Applicant indicates on page 20, 3rd parag. of Applicant's response, December 20, 2005, that, "it is in our submission sufficient for an applicant to describe how a claimed invention can be made. There is in our submission no obligation on an applicant to describe how to succeed also using methods that may be more problematic and less desirable than those disclosed." In response, the issue at hand is that the claims broadly encompass a wide variety of methods in which "a reduced functional extent" of a chloride channel is achieved. In addition to a method of targeted gene disruption via homologous recombination could be used, the claim also encompasses methods such as antisense to reduce levels of mRNA transcript or use of a weakly expressing promoter to drive low amounts of a gene of interest. The art indicates that either of these methods is unpredictable (e.g. see Agrawal and Kanimalla reference and Goswami reference). While the specification teaches one aspect that is used to obtain cells that do not express a gene of interest (i.e. homologous recombination), this one aspect does not enable the full breadth of the genus of possible ways that this can be achieved. As such, the rejection regarding this issue is maintained.

For this reason, the specification and the art do not provide guidance for an artisan to arrive at the claimed invention.".

Applicant respectfully disagrees for the following reasons:

1) The Examiner has maintained rejection against all of the claims on the ground of lack of enablement. The Examiner indicates that the claims are enabled for isolated mouse or human muscle cells or a cell line comprising a disruption of endogenous ClC-1 and a cell membrane preparation or cell vesicle obtained from such a cell or cell line and also for various other cells and cell lines involving disruption of ClC-Kb, ClC-5 or ClC-7. The Examiner maintains that the specification lacks enablement in respect of cells, cell lines or cell membrane preparations or cell vesicles comprising disruption of other individual chloride channels, multiple disruptions of chloride channels, over expression of chloride channels and methods of using such cells, cell lines, membrane preparations or vesicles.

The objection is a somewhat unusual one in that although on page 5, the Examiner sets out classes 1 (a) to (g) and 2 (a) to (g) of cells and cell components that are said not to be enabled, it would not appear to be the Examiner's contention in respect of the making of the cells that the basic techniques needed for producing cells with single or plural gene knockouts or with over expression of single or multiple genes are unknown or inapplicable to cells of interest in respect of the invention. Rather, the Examiner's reasons for this objection are couched in terms of lack of predictability of phenotypes.

For the record however, the techniques needed for producing gene knockouts and for producing over expression are well known and are described in common text books such as 'Principles of Gene Manipulation and introduction to Genetic Engineering', Editors R. W. Old and S. B. Primrose 1994, Blackwell Science Ltd. The sequences of the relevant chloride channels were known in the art. Should any of this be in dispute (and we think that it is not), then we can provide further references.

However, we note that the Examiner acknowledges that homologous recombination is a known method for generating targeted disruptions in a gene of interest, but remarks that the art teaches that phenotypes exhibited by mice with gene disruptions are not predictable.

The rejected claims relate to cells or cell preparations and their use. They do not relate to mice. It is therefore the submission of the applicant that the phenotype of mice having the

relevant gene disruptions is simply an irrelevance and whether said phenotypes are predictable is still more so.

The Examiner has further remarked on page 9, that a phenotype exhibited in a mouse may not be readily applicable to the human condition. This again, in our submission is an irrelevance, having regard to what is actually claimed.

The rejected claims are directed in the first instance to cells and cell components. The Examiner alleges on page 10 that the ability of the skilled worker to use such cells depends on a known relationship between the gene of interest and the human condition. The Examiner argues that 'The enabled use of the cell hinges upon the fact that the cell in an *in vitro* assay stems from the understanding of what biological characteristics the specific chloride channel has with pathology of a human disease. Subsequently, because ClC-4 and ClC-6 have no known pathology associated with them..., the use of cells comprising disruptions in these channels is not enabled'.

However, the invention claimed is not concerned with investigating compounds relevant to any condition in the mouse or human mediated by expression of ClC-4 or ClC-6, or of other chloride channels than ClC-7.

The specification sets out that there is a relationship in the human between that ability of osteoclasts to resorb bone and the action of the chloride channel ClC-7 in the osteoclasts. As has been submitted by the Applicant in earlier correspondence, the claimed cells and methods are useful in the search for effective and specific modulators of ClC-7 function. In order to develop such effective ClC-7 function modulators it is useful to have cells in which ClC-7 function is enhanced, so that one can test compounds for decreasing the chloride channel transport properties of the cell. However, to determine that promising compounds are specific for action on ClC-7, it is useful to be able to test them on cells that are enhanced in or deficient in activity of other chloride channels.

By way of example, discovering that a given compound decreases chloride channel transport when ClC-7 is enhanced, but not when ClC-7 is knocked out shows that the compound may be a candidate for a specific ClC-7 modulator. Testing the compound on cells in which ClC-4 (say) is knocked out may reveal that the chloride channel transport modulating activity of the test compound is unaffected by the presence of ClC-4, which will further confirm the likelihood of the compound being ClC-7 specific. On the other hand, it may show that the modulating action of the test compound is heavily ClC-4 dependent, leading to the conclusion that even if the compound does modulate ClC-7 transport, it does not do so specifically, but is active against other chloride channels.

Thus, the ClC-4 knock out cells are useful, whether a mouse having the ClC-4 knock out exhibits a distinct phenotype or not and whether there is any similarity between the phenotype of the mouse and the phenotype of a human having a similar genetic trait.

These arguments have been put before the Examiner previously (Pages 11-12, Response of 18th November 2005), but we cannot see that any note has been taken of them. Nothing in the lengthy exposition from the Examiner on pages 4 to 10 of the official action explains why cells having a deletion of, for example, ClC-4 will not be useful in the manner described above for exploring the specificity of potential ClC-7 modulators. Nor is it explained why such utility would depend on knowing in advance the phenotype of a ClC-4 deficient mouse or human.

Whilst the Examiner has explained at length why it is considered that the effect on phenotype in the mouse and relationship to human disease of knocking out certain chloride channels is unknown, the Examiner has not explained what relevance this has to the utility described by the Applicant. The Examiner has asserted that 'the enabled use of the claimed cell hinges upon the fact that the cell in an *in vitro* assay stems from the understanding what biological characteristics the specific chloride channel has with pathology of a human disease.' However, no justification for that assertion is provided dealing with the specific utility in finding effective and specific modulators of ClC-7 activity which the Applicant has previously explained.

2) Similarly, from pages 11 to 12 of the Official Action, the Examiner sets out the basis for an objection of lack of enablement in relation to over expression of various ClC channels which is couched in terms similar to those of the objection to the enablement of cells having deletions. Again, it is said that the art teaches no human condition associated with over expression of any chloride channel and that over expression does not result in an animal with a predictable phenotype. From this it is deduced by the Examiner that 'an Artisan cannot predict that over expression of a gene of interest results in a transgenic animal exhibiting any phenotype and whether that animal is a model for a human disease or condition'.

Once again, this objection fails to substantiate that the cells and methods claimed are not useful for the purpose which the Applicant has described, which is in no way dependent on the effect on phenotype of over expressing chloride channels. We reiterate, the purpose of over expressing chloride channels in the Applicant's invention is to allow knowledge to be gained through screening compounds so as to determine whether compounds are effective and specific modulators of ClC-7 transport. Whatever the phenotype of a ClC-4 over expressing mouse or human may be, cells which over express ClC-4 are useful in that if a test compound strongly modulates chloride channel transport activity in such a cell, it can be eliminated in the search for a ClC-7 specific modulator.

3) It has further been objected that there a lack of enablement of cells that comprise multiple over expression and/or disruption of chloride channels (page 13 of the Official Action).

As regards multiple over expression constructs, the Examiner reasons that the art teaches that resulting phenotypes will be unpredictable. From this the Examiner deduces that 'an artisan cannot predict what phenotype of a disease would result in cells expressing multiple transgenes, so the artisan is not enabled to use the cells in any assay'.

In our submission, the last clause in the Examiner's sentence quoted above is simply a *non-sequitur* . There is simply no need to associate multiple over expression with any disease

phenotype in order to practise the invention for the purposes described above, i.e. to test candidate compounds for their ability to modulate the ClC-7 channel effectively and specifically. In such a process, the phenotype engendered by multiple over expression is not of any interest. One simply wishes to ascertain whether a test compound affects chloride channel transport in a manner that is consistent with or inconsistent with it affecting only the ClC-7 channel.

4) Also on page 13, the Examiner raises a similar argument in relation to cells having multiple deletions. The answer is the same. Cells with multiple deletions are useful for deducing which ClC channels a candidate compound modulates and which it does not. Such utility is unconnected with the phenotype resulting from the multiple deletions.

5) On page 14, the Examiner asserts that 'in the light of these issues of unpredictability of phenotype in making cells expressing a transgene or making cells comprising a disruption of a gene of interest, an artisan cannot reasonably know how to make cells comprising a combination of disruptions and over expression constructs.

In our submission, the rejection is not supported by the Examiner's premises. The making of the modified cells is not shown by the Examiner's reasoning to be dependent in any way on the predictability of the resulting phenotype. Techniques for making cells having multiple gene disruptions or having multiple gene expression enhancements are well known in the art. Such methods are not dependent (and have not been shown by the Examiner to be dependent) on advance knowledge of the phenotype that will be engendered. There is simply no logical connection between the Examiner's statements regarding lack of phenotype predictability and the Examiner's conclusions that the relevant cells cannot be made. Furthermore, in our submission, the very fact that such cells can be made is fully sufficient to justify the enablement provided in relation to the claims to cells and cell components, since such claims are fully enabled once the description has allowed the claimed cell to be made. That is true quite independently of whether the specification also discloses ways of using the cells once made (although in this case of course, the specification does also describe and enable uses for the cells).

6) Starting with the last paragraph on page 14, the Examiner objects that the specification embraces many methods of providing reduction of expression of ClC genes, not only homologous recombination, but also antisense methods. The art is said to show that antisense methods are unpredictable.

The claims are not of course directed to methods of making cells with changes in their chloride channel activities. The rejection is therefore fundamentally inappropriate. Claims to things, be they chemical entities, machines or cells, are not normally limited to specific methods of production, irrespective of the number of methods of making them that the specification enables. Thus, where a new chemical entity it claimed, it is considered to be an enabling disclosure supporting such a claim if one provides one method of synthesizing the compound. One does not get the USPTO insisting on the granted claim being limited to the described method of production. That is so even if there are alternative synthetic routes that the Examiner can deduce may involve problems.

In the case of a new machine, one again does not experience rejections to claims to the machine per se, not limited to a specific method of production.

Here too, there is no reason why a claim to a cell per se should require limitation by reference to its historical method of production, or rather the method by which its ancestors were produced.

The Examiner has answered the Applicant's previous submission on this point by stating 'In response, the issue at hand is that the claims broadly encompass a wide variety of methods in which 'a reduced functional extent' of a chloride channel is achieved'. This is of course basically incorrect. The claims do not encompass methods for reducing chloride channel activity at all. They are not directed to such methods any more than a claim to a new chemical

entity encompasses methods of making such a compound or a claim to a new machine encompasses methods of making it.

The rejection is moreover out of line with previous practice of the Office. For instance, Claim 1 of US6562588 reads:

1. A process for the production of a glycoprotein, comprising expressing a nucleic acid encoding the glycoprotein in a Chinese Hamster Ovary cell line, wherein a constitutive sialidase gene of the Chinese Hamster Ovary cell line is not functionally expressed.

That is a claim which like those of the present application is directed to a method not of making a knock out cell, but of using one. It has been granted without restriction as to the way in which the knock out cells were produced.

Claim 3 of the same case reads:

3. A recombinant eukaryotic cell line wherein a constitutive sialidase gene of the cell line is not functionally expressed and wherein the cell line is Chinese Hamster Ovary cell line.

This illustrates that objections of the kind raised by the Examiner are not customary against claims to cell lines either. Applicant respectfully requests reconsideration.

3. Claims 42-67 are newly rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

> The Examiner states, "The claims use the term, "functionally expressed." It is unclear what this term means. Does "functionally" refer to "expressed," which means that the invention being envisioned is an overexpression construct? Or, does "functionally" refer to the chloride

> channel? Nothing in the specification provide any guidance as to what this term is. Similarly, the claims use the term "preferentially functionally expressed." While the specification indicates that "preferentially" means "predominantly" (specification, page 5, 2nd parag.), it is unclear what is meant by "predominantly functionally expressed". Also, the claims use the phrase, "reduced functional extent". Does this phrase refer the levels of expression or to the activity of the chloride channels? The method claims depend on the claimed cells and are thus included in the rejection.".

Applicant respectfully disagrees. Claims have been rejected as being indefinite on the basis of the use of the term 'functionally expressed'. However, it has not been explained why really this term is thought to be unclear. Clearly, a channel is functionally expressed if it is (a) expressed and (b) the form in which it is expressed is such that it is functional. It is not therefore functionally expressed if it is not expressed at all and it is not functionally expressed if it is expressed in a modified form which is not functional.

The Examiner asks whether the term 'functionally' relates to the expression or to the chloride channel, but this is a false antithesis. Separate from consideration of the chloride channel, it would be meaningless to enquire whether expression was functional or not. Clearly, the functionality of the expression can be judged only in terms of whether the resulting chloride channel is functional.

Moreover, the expression is one which is much used and is clearly understood in the art. For instance, searching the truncated phrase 'functionally express*' on the PubMed database yields over 18,000 hits and searching the full text of granted US Patents for the phrase 'functionally expressed' yields over 400 hits. US6008437 is an example where the term is used extensively in the claims. US6562588 is another.

Secondly, the Examiner has raised objection to the term 'preferentially functionally expressed', stating that it is unclear, without explaining why it should not be considered clear on its face. We submit that it is clear on the face of the words that if a first named channel is preferentially expressed with respect to a second named channel, there is to be a greater

expression of the first than of the second. Again, what counts is functional expression, in that expression of a non-functional version of the channel is not relevant.

Thirdly, the Examiner has objected to the term 'reduced functional extent'. It is queried whether this relates to the level of expression or the activity of the chloride channels. Again, we submit that the antithesis proposed by the Examiner is false and that the term is clear on its face. If the channel is expressed to a reduced functional extent, one finds that the activity of the channel is reduced either because it is expressed to a reduced extent or because its functionality has been reduced, or both. Given that the art shows that the term 'functionally expressed' is well understood and widely used, there is no reason for objection to be taken to the term 'reduced functional extent'. Applicant respectfully requests reconsideration.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. Applicant's representative would like to discuss this case with the Examiner to learn if any outstanding issues remain after consideration of this Amendment. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record. The Applicants believe that a three-month extension of time is required.

The Applicants conditionally petition for a further extension of time to provide for the possibility that such a petition has been inadvertently overlooked and is required. As provided below, charge Deposit Account No. **04-1105** for any required fee.

Date: November 22, 2006

Respectfully submitted,

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